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Caries infiltrant combined with conventional adhesives for sealing sound enamel in vitro

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Abstract: **OBJECTIVE:** To test the null hypothesis that combining low-viscosity caries infiltrant with conventional adhesive resins would not improve sealing of sound enamel against demineralization in vitro. **MATERIALS AND METHODS:** Bovine enamel discs (N = 60) with diameter of 3 mm were randomly assigned to six groups (n = 10). The discs were etched with 37% phosphoric acid for 30 seconds and treated with resins of different monomer content forming the following groups: (1) Icon (DMG), (2) Transbond XT Primer (3M ESPE), (3) Heliobond (Ivoclar Vivadent), (4) Icon + Transbond XT Primer, and (5) Icon + Heliobond. Untreated etched samples served as the negative control. Specimens were subjected to demineralization by immersion in hydrochloric acid (pH 2.6) for 80 hours. Calcium dissolution into the acid was assessed by colorimetric analysis using Arsenazo III method at 16-hour intervals. Groups presenting high protection against demineralization were subjected to further acidic challenge for 15 days with calcium measurements repeated at 24-hour intervals. Data were analyzed by Kruskal-Wallis test and Mann-Whitney U-test. **RESULTS:** Untreated specimens showed the highest amount of demineralization. Icon and Transbond XT primer decreased the mineral loss significantly compared to the control. Heliobond performed significantly better than both Icon and Transbond XT primer. Combination of Icon both with Transbond XT primer or Heliobond served as the best protective measures and maintained the protective effect for the additional 15-day acidic challenge. **CONCLUSIONS:** Within the limitations of this in vitro study, it could be concluded that the use of low-viscosity caries infiltrant prior to application of the tested conventional adhesives increases their protective effect against demineralization.

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Caries Infiltrant combined with conventional adhesives for sealing sound enamel *In Vitro*

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Color Figures – yes or no?

No

ABSTRACT

Objective: To assess the preventive effect of a low-viscosity caries infiltrant, two conventional adhesives and their combinations against demineralization *in vitro*.

Materials and Methods: Bovine enamel discs (N=60) with diameter of 3 mm were randomly assigned to 6 groups (n=10). They were etched with 37% phosphoric acid for 30 seconds and treated with resins of different monomer content forming the following groups: 1) Icon (DMG); 2) Transbond XT Primer (3M ESPE); 3) Heliobond (Ivoclar Vivadent); 4) Icon + Transbond XT Primer and 5) Icon + Heliobond. Untreated etched samples served as the negative control. Specimens were subjected to demineralization by immersion in hydrochloric acid (pH 2.6) for 80 hours. Calcium dissolution into the acid was assessed by colorimetric analysis using Arsenazo III method at 16-hour intervals. Groups presenting high protection against demineralization were subjected to further acidic challenge for 15 days with calcium measurements repeated at 24-hour intervals. Data were analyzed by Kruskal-Wallis and Mann-Whitney tests.

Results: Untreated specimens showed the highest amount of demineralization. Icon and Transbond XT primer decreased the mineral loss significantly compared to the control. Heliobond performed significantly better than both Icon and Transbond XT primer. Combination of Icon both with Transbond XT primer or Heliobond served as the best protective measures and maintained the protective effect for the additional 15-day acidic challenge.

Conclusion: Within the limitations of this *in vitro* study, it could be concluded that the use of low-viscosity caries infiltrant prior to application of the tested conventional adhesives increases their protective effect against demineralization.

Key words: Sealing; White spot lesion; Caries infiltrant

INTRODUCTION

Fixed orthodontic appliances create retentive areas that are difficult to clean mechanically which leads to plaque accumulation. Daily-consumed carbohydrates are fermented by the bacteria that were colonized in the plaque and lead to decreases in the intraoral pH.¹ This results in dissociation of calcium (Ca) and phosphate (PO₄) ions from enamel in an attempt to reach chemical equilibrium in the oral environment.^{1,2} Thus, one possible inherent problem during the whole course of orthodontic treatment is the subsurface enamel demineralization around brackets, representing the primary phase of caries formation.²

Prevalence of these acquired surface lesions due to orthodontic treatment, so called white spot lesions (WSL), is relatively high, affecting more than forty to sixty per cent of the patients.^{3,4} They can appear very rapidly, as fast as in a couple of weeks after the placement of brackets.⁵ The efficacy of preventive measures against this phenomenon has been questioned during the last two decades.^{2,6} Prevention methods mainly target the remineralization process and the inhibition of present bacterial flora through topical fluoride applications, use of adhesives with remineralization potential that contains amorphous calcium phosphate or fluoride, casein phosphopeptide-amorphous calcium-phosphate containing pastes, chlorhexidine mouth rinses, ozone applications, probiotics, xylitol and sealants.^{2,6,7} Although remineralization of WSL could be achieved to some extent using related measures, clinically it has been shown that WSL do not disappear unless they are removed mechanically by abrasion, etching, **masking by resin infiltration** or treated in a restorative fashion.^{8,9} Among these, prevention measures that do not require patient compliance are considered to be more predictable since only 13% of the patients were reported to achieve excellent cooperation with the use of mouthrinses and tooth brushing.^{2,10} Therefore, sealing the susceptible enamel prior to bracket bonding in order to form a caries-protective shield has been the focus of interest in previous studies that primarily intend to eliminate patient compliance.^{7,11}

In principle, sealants cover the whole buccal surface adjacent to brackets, forming a physical barrier.^{2,7,11} This protective shield is subjected to physical challenges such as acid attacks from bacterial plaque and acidic soft drinks as well as daily tooth brushing, which might impair the

seal.^{5,11} Recently, a low viscosity caries infiltrant with high penetration ability has been shown to increase the protective capacity of conventional adhesives against demineralization.¹² This new resin has been originally developed to arrest WSL progression and to prevent further demineralization by forming a resistant condensed layer via infiltrating the demineralized enamel.¹³ In contrast to conventional sealants, where the physical barrier remains on the enamel surface as a covering coat, this infiltrant presents rapid capillary penetration into the pores creating a diffusion barrier within the enamel with very low-viscosity and superior surface wetting abilities. In addition, new retention areas for plaque accumulation at the sealed margins are being avoided.^{12,13} However, in spite of the deeper penetration of carious lesions where there is a porous structure for the resin to infiltrate, it has not been shown if this resin can infiltrate phosphoric acid etched sound enamel where only limited capillary diffusion is imaginable.

Therefore, the objective of this study was to evaluate the effect of low viscosity adhesive resin applied after phosphoric acid etching with and without subsequent use of conventional bonding agents on sealing of sound enamel. The null hypothesis tested was that combining the low-viscosity caries infiltrant with conventional adhesive resins would not improve sealing of sound enamel against demineralization.

MATERIALS AND METHODS

Bovine enamel, two conventional adhesive resins (Transbond XT Primer and Heliobond) and a low-viscosity caries infiltrant (Icon) were employed in this *in vitro* study. The adhesives used were conventional bonding agents with different ratios of Bis-phenol-A-glycidylmethacrylate (Bis-GMA) and Triethyleneglycol dimethacrylate (TEGDMA) content. The low-viscosity caries infiltrant was TEGDMA based. The chemical compositions of the materials are summarized in Table 1.

Study design

Specimens were divided into 6 groups (n = 10): unsealed control, caries infiltrant, conventional orthodontic bonding agent, unfilled bonding agent, caries infiltrant + conventional orthodontic bonding agent, caries infiltrant + unfilled bonding agent. All specimens were subjected to 80-hour acid challenge for demineralization. Calcium release was measured every 16 hours using Arsenazo III colorimetric analysis method.¹⁴ The groups presenting almost absolute protection were kept under acidic attack for another 15 days with calcium measurements performed every 24 hours.

Specimen Preparation

Bovine incisors (N=10) stored in 0.5% chloramine solution at 4°C no longer than 6 months were initially cut from their roots. Six discs of enamel with a diameter of 3 mm were cut from the labial aspect of each tooth using a custom-made diamond-coated trephine (80 µm, Intensiv SA, Lugano-Grancia, Switzerland). The discs were then flattened from the bottom to approximately 2 mm in height (Struers, Birmensdorf). Each piece was randomly assigned to 6 groups assuring equal distribution of incisal and gingival sections per group. They were embedded with their labial surfaces exposed in auto-polymerizing acrylic resin (Paladur, Heraeus Kulzer, Wehrheim, Germany) in cylindrical moulds (6 mm diameter and 3 mm thickness). The embedded specimens were ground flat and polished with water-cooled carborundum discs (1200, 2400 and 4000 grit, Struers, Erkrat, Germany). The samples were stored in distilled water (grade 3) until sealing procedures.

Sealing Procedure

All specimens were etched with 37% H₃PO₄ (Orbis Dental, Munster, Germany) for 30 s and rinsed with water for 30 s. After air-drying, the specimens except the unsealed control group were treated as follows:

- 1) Caries infiltrant: Ethanol (Icon-Dry, DMG, Hamburg, Germany) was applied for 30 s and dried for 10 s. The low-viscosity caries infiltrant (Icon infiltrant, DMG, Hamburg, Germany) was applied in one coat with a micro-brush, let set for 180 s, light-cured for 60 s, a second layer was applied, let set for 60 s and light-cured for 40s.

- 2) Conventional orthodontic bonding agent: Adhesive primer (Transbond XT Primer, 3M Unitek, Monrovia, USA) was applied in one coat with a micro-brush and light-cured for 60 s.
- 3) Unfilled bonding agent: Adhesive (Heliobond, Ivoclar Vivadent, Schaan, Liechtenstein) was applied in one coat with a micro-brush and light-cured for 60 s.
- 4) Caries infiltrant + conventional orthodontic bonding agent: Steps 1 and 2 were repeated subsequently.
- 5) Caries infiltrant + unfilled bonding agent: Steps 1 and 3 were repeated subsequently.

All procedures involving air/water syringe and light curing unit were performed using a custom made device assuring standard distance to the specimens from the application tips (air/water syringe and light curing unit) and standard pressures provided by the air/water syringe. The specimens were photo-polymerized using the Bluephase G2 curing unit (Ivoclar Vivadent, Schaan, Liechtenstein; output intensity: 1220 mW/cm² at light-guide tip; 1110 mW/cm² at 3 mm). The irradiance was measured using a CC3-UV detector and USB 4000 spectroradiometer (Ocean Optics, Dunedin, FL, USA). Before and after each pretreatment group, the light-curing unit was checked for constant output. Following these pretreatments, the specimens were stored in distilled water for 24 hours at 37°C for complete polymerization.

Acidic challenge and evaluation of sealing ability

All specimens were immersed in hydrochloric acid (2500 µmolH⁺/l, pH 2.6) under constant motion for 80 hours. Groups providing almost absolute protection against demineralization were further challenged for an additional 15-day period. Sealing ability was quantified by the amount of Ca released from the specimens into the acid solution in a flat micro-plate reader (Molecular Devices, Ismaning/Munich, Germany) using Arsenazo III method as previously described by Attin *et al.*¹⁴. Arsenazo III reacts with calcium to form a bluish-purple complex. The intensity of the color complexes is proportional to the calcium concentration and can be determined photometrically by the micro-plate reader according to Beer-Lambert law. Ten micro-liters of the acid solution was added to the wells of the micro-titre plate and mixed with 100 micro-liters of the color reagent (Fluitest CA AIII R1, Biocon Diagnostik, Vöhl/Marienhagen,

Germany). Absorbance was read at 650 nm in the micro-plate reader. The measurements were performed at a room temperature of 25 °C.

Statistical Analysis

A sample size of 10 in each group was calculated to have 90% power to detect a difference in means of 9.5 µmolCa/ml. This assumes that the Group 1 standard deviation is 4.2 and the Group 2 standard deviation is 5.5 using a two group Satterthwaite t-test with a 0.01 two-sided significance level. The approximate normal distribution was investigated by Kolmogorov – Smirnov and Shapiro – Wilk tests. As the data were not normally distributed, Kruskal-Wallis test was applied to analyze possible differences between the groups at different time points. This was followed by Mann-Whitney test, separately for all combinations of two group comparisons. The level for significance was set at $P<.05$.

RESULTS

Calcium Dissolution

At each time point, Kruskal-Wallis test revealed significant differences between the groups. Mean calcium dissolution into the acid for each group every 16 hours and cumulative Ca dissolution after 80 hours are presented in Table 2 and Figure 1, respectively.

Untreated specimens presented the highest rate of Ca release over the whole experiment period, whereas the combination of caries infiltrant and the conventional adhesives presented almost complete sealing of the surface against demineralization at all times. The application of caries infiltrant and conventional orthodontic adhesive (Transbond XT primer) alone reduced the amount of Ca dissociated significantly when compared to the untreated specimens but significantly inferior compared to the unfilled conventional restorative adhesive (Heliobond) alone. The groups providing almost complete protection (Icon + Transbond XT primer and Icon + Heliobond) maintained the seal during the additional testing period and showed no increase in the amount of Ca released into the acid solution for 15 days representing approximately 7 months (Figure 2).

DISCUSSION

In this *in vitro* trial, the low-viscosity caries infiltrant demonstrated similar sealing ability to the conventional orthodontic adhesive on sound enamel. The unfilled bonding agent with the highest viscosity was superior compare to the aforementioned groups when applied alone. The protective potential of conventional adhesives against enamel demineralization was improved when combined with the caries infiltrant. Since the sealing effect obtained in groups with the caries infiltrant application presented significant differences, the null hypothesis is rejected. This also implied that the application of the caries infiltrant following 37% phosphoric acid etching on sound enamel prior to orthodontic bonding could be an alternative to be used as an additional preventive measure against WSL formation.

Etched enamel surfaces adjacent to orthodontic brackets are usually covered with primer or the bonding agent that is used to wet the surface for better formation of resin tags for mechanical interlocking.⁵ This thin layer seals the mineralized tissue underneath against acidic challenges resulting from dietary intake of carbohydrates and soft drinks. The daily frequency and magnitude of these pH drops depend on many variables such as frequency of sugar intake, percentage of sugar in the food, properties of saliva and intraoral flora, which shows a great diversity among individuals.¹⁵ In this present study, the acid attack was applied continuously mimicking an estimated time period of six to seven weeks with 5 pH drops of 20 minutes per day. Groups providing almost complete protection against Ca loss were further subjected to acid challenge for 15 days representing approximately seven month, summing up to 9 months. The pH of the acid used was significantly lower than that of the organic acids produced by bacteria. This lower pH was used to increase the quantity of Ca dissolved to generate detectable amounts in short time periods and to assure the duration of acidic challenge to represent at least the estimated time period. It was assumed that the sealants performing well under these highly demineralizing conditions would also be able to show the same relative protective effects against demineralization caused by weaker acids. One limitation regarding the demineralization cycle might be that no remineralization by saliva or other regular protective measures such as the use of fluoride containing toothpaste has been applied. The rationales behind this approach were to increase the precision of the measurement method by eliminating

possible Ca contamination from the toothpaste and avoid possible interactions between measurements. A secondary objective was to simulate the worst-case scenario for demineralization without the presence of preventive measures. With these aspects in mind, the endurance of the protective effect provided by Icon + Transbond XT primer and Icon + Heliobond, was anticipated to last throughout the whole course of orthodontic treatment since the seal didn't present any signs of impairment at the end of the 15-days challenge representing approximately 9 months *in vivo*.

One important prerequisite in sealing enamel is the high surface wettability property of the applied resins.¹² High TEGDMA content and ethanol in adhesives were shown to increase the capillary penetration and wetting ability of the resins facilitating better micro-mechanical unity with the enamel whereas Bis-GMA content decreases this property, which might result in weakened plugging of the porosities.^{13,16,17} On the other hand, the high TEGDMA content in the resin matrix increases polymerization shrinkage and stress resulting in lower physical properties. Similarly, more oxygen inhibition and polymerization shrinkage of the low-viscosity caries infiltrant was reported to create heterogeneous areas within the penetrated material, resulting in insufficiently filled porosities of the surface.^{17,18} In that respect, Icon with the highest TEGDMA content among the tested resins was expected to provide better penetration into the enamel with higher contact area. In addition, voids in sealant surface due to the oxygen inhibition and polymerization shrinkage were anticipated. As expected, Icon and Transbond XT primer presented reduced Ca dissolution compared to the untreated samples, but performed inferior compared to Heliobond, which was predicted to form a homogenous surface covering owed to higher Bis-GMA content.

On the other hand, wetting of the enamel with Icon prior to Heliobond or Transbond XT primer performed better than all the single applications and provided almost complete sealing. It might be suggested that the lacking ability of capillary penetration of the two more viscous resins was compensated by Icon resulting in a highly protective layer against demineralization.

There is no study in the literature evaluating the effect of Icon on enamel sealing following phosphoric acid etching. However, Schmidlin *et al.*¹¹ reported a similar reduction effect on apatite dissolution following hydrochloric acid etching on both sound and demineralized enamel recently. In particular, Heliobond alone and its combination with Icon performed superior than the infiltrant application alone as found in the present study. In contrast to the present findings, combining Icon with Heliobond did not provide better protection than Heliobond alone. This might be attributed to the extensive etching effect of 120 s hydrochloric acid application, which was primarily intended to create a permeable outer layer in the presence of WSL.¹⁹

Penetration time is another important factor determining the rate of resin impregnation and plugging the gaps formed by etching.²⁰ Icon was the only resin with prolonged penetration time where as Heliobond and Transbond XT primer were photo-polymerized right after their application on the etched surface. It may be suggested that this was also another factor increasing the sealing property. However, a 180-second application time might not be easy to obtain in clinical practice, especially on the buccal surfaces of posterior teeth when the patient is in a supine position. Still, allowing the resin to penetrate as long as possible prior to photo-polymerization should be acknowledged as an improving factor.²⁰

The resistance of the test groups to mechanical wear was not addressed in this study in order to isolate acidic challenge as the only factor affecting the sealant capability. The wear resistance of the groups presenting protective effect might be the interest of future studies.

CONCLUSIONS

Within the limitations of this study, the following could be concluded:

- The low-viscosity caries infiltrant (Icon) and the conventional orthodontic bonding agent (Transbond XT primer) reduced enamel demineralization when applied alone.
- The conventional bonding agent (Heliobond) with higher viscosity provided higher protection compared to the conventional orthodontic bonding agent and the caries infiltrant when applied alone.

- Both conventional bonding agents provided almost absolute sealing when combined with the low-viscosity caries infiltrant.

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FIGURE LEGENDS

Figure 1. Cumulative calcium dissolution in $\mu\text{mol/ml}$ after 80 hours. Groups that are not significantly different are marked with same capital letters.

Figure 2. Daily calcium dissolution (mean and SD) in groups presenting protective effect showing maintenance of the seal.

Figure 1. Cumulative calcium dissolution in $\mu\text{mol/ml}$ after 80 hours. Groups that are not significantly different are marked with same capital letters.

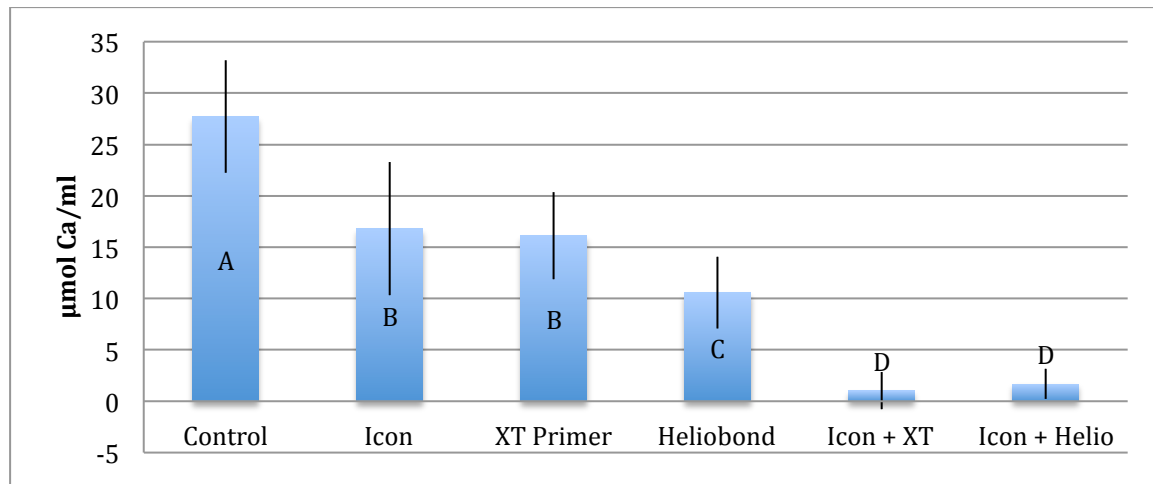


Figure 2. Daily calcium dissolution (mean and SD) in groups presenting protective effect showing maintenance of the seal.

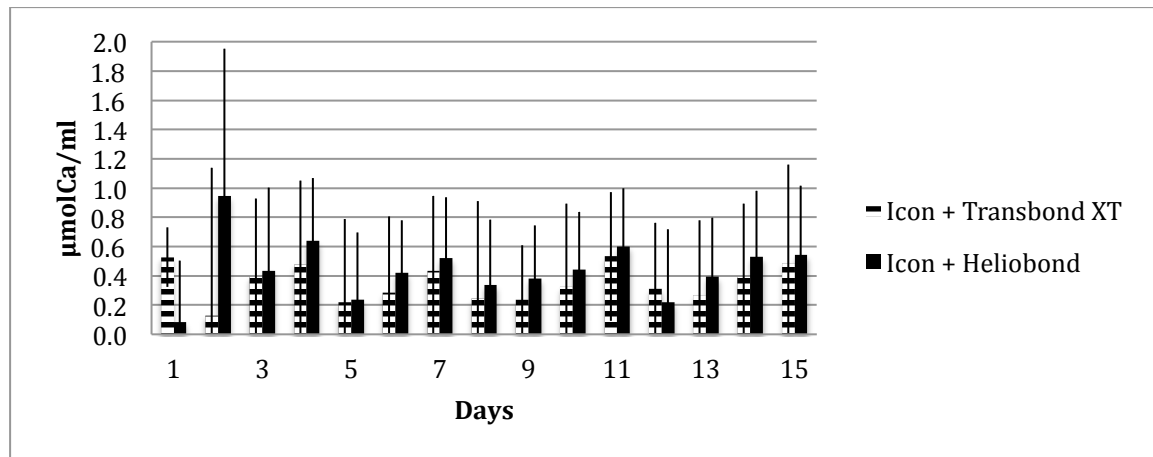


Table 1. Composition of the low-viscosity caries infiltrant and the conventional bondings resins according to the manufacturers' information.

Product	Chemical composition	Manufacturer
Icon (Infiltrant)	TEGDMA based resin matrix, Initiators - additives	DMG, Hamburg, Germany Batch no. 634902
Transbond XT Primer	bis-GMA 45-55 % TEGDMA 45-55 % 4-dimethylaminobenzene ethanol <0.5 % camphorquinone <0.3 % hydroquinone <0.03 %	3M Unitek, Monrovia, USA Batch no. 290791
Heliobond	bis-GMA 50-100% TEGDMA 25-50 % Initiators - stabilizers	Ivoclar Vivadent, Schaan, Liechtenstein Batch no. 30513
bis-GMA: Bis-phenol-A-glycidylmethacrylate; TEGDMA: Triethyleneglycol dimethacrylate		

Table 2. Mean \pm (SD) calcium dissolution ($\mu\text{mol/ml}$) every 16 hours. Comparisons at each time point of measurement that are not significantly different are marked with the same capital letters. (Read vertically)

Groups	Time Points (hours)				
	16	32	48	64	80
Control	10.27 A	7.24 A	4.59 A	3.17 A	2.46 A
	(1.83)	(1.63)	(0.80)	(0.64)	(0.34)
Icon	6.06 B	4.92 B	2.13 B	1.97 B	1.74 B
	(1.53)	(1.12)	(0.49)	(0.56)	(0.24)
Transbond XT Primer	4.99 B	5.05 B	2.34 B	1.96 B	1.78 B
	(3.02)	(2.00)	(0.86)	(0.70)	(0.48)
Heliobond	3.88 B	2.99 C	1.36 C	1.21 C	1.16 C
	(0.46)	(0.44)	(0.26)	(0.23)	(0.12)
Icon + Transbond XT Primer	0.31 C	0.33 D	0.14 D	0.15 D	0.11 D
	(0.63)	(0.53)	(0.23)	(0.36)	(0.41)
Icon + Heliobond	0.43C	0.46 D	0.27 D	0.25 D	0.26 D
	(3.59)	(1.45)	(1.75)	(1.18)	(0.37)